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EFFECTS OF INSPIRED CO_2 AND BREATHING RESISTANCE ON NEUROCOGNITIVE AND POSTURAL STABILITY IN U.S. NAVY DIVERS

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14. ABSTRACT: **Purpose:** The aim of this investigation was to evaluate the effects on neurocognitive performance and postural stability of inspired CO_2 and breathing resistance during endurance exercise. **Methods:** Subjects (N=16) completed five dives. Each dive involved a different combination of inspired CO_2 (0%, 1%, & 2% SEV CO_2) and breathing resistance (work of breathing per tidal volume of 1.0 kPa and 1.8 kPa at a minute ventilation of 62.5 L/min. Neurocognitive functioning was assessed pre/post each dive using a subset of Automated Neuropsychological Assessment Metrics (ANAM). Postural stability was assessed post each dive through the use of a Wii Balance Board. Amplitude and sample entropy (SampEn) were calculated for the center of pressure (COP) in both the anterior-posterior (X) and medial-lateral (Y) directions. A general linear model multivariate analysis of variance (GLM MANOVA) with repeated measures was conducted to evaluate the effect of exercise and breathing conditions on ANAM performance. Four GLM MANOVAs with repeated measures were conducted to evaluate the effect of breathing conditions on postural stability. Alpha was set at p = 05. **Results:** There were no significant differences found. **Conclusions:** The results of this study can be interpreted as the various combinations of high CO_2 levels and high breathing resistance did not impair neurocognitive performance and postural stability. Future studies should focus on other neurocognitive and neurophysiological tests to verify these results. However, it should be noted that the individuals who were most likely to have shown neurocognitive effects from the CO_2 exposures did not complete post-exercise neurocognitive assessments.

15. SUBJECT TERMS: neurocognitive, ANAM, balance, CO_2, carbon dioxide, hypercapnia, breathing resistance, hyperoxia, UBA, underwater exercise

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CONTENTS

TABLES

INTRODUCTION

Hypercapnia, elevated arterial carbon dioxide (CO_2) partial pressure (P_aCO_2), may result from CO_2 buildup in the underwater breathing apparatus (UBA) breathing loop and subsequent retention within the tissue of the body. Inadequate ventilation (volume of air inhaled per minute) by the diver or failure of the CO_2 absorbent canister to remove CO_2 from the exhaled gas will cause a buildup to occur. Common symptoms of minor hypercapnia are increased depth and rate of breathing, labored breathing, headache, dizziness, and mental confusion.[1,2] More severe hypercapnia can result in adverse cognitive and psychomotor performance and even unconsciousness.[3-5]

Military diving operations often involve the use of a closed or semi-closed circuit rebreather UBA, which removes CO_2 from expired gas, and allows a diver to reuse the expired gas repeatedly. Rebreather UBAs recirculate expired gas around a breathing loop which consists of breathing hoses, one-way values, CO_2 absorbent (i.e., scrubbers), and a counter-lung. Additional gas is added to the UBA to replace the oxygen consumed by the diver.[6,7] Assuming that the CO_2 absorbent is functioning properly and CO_2 is not accumulating within dead space, the inspired CO_2 partial pressure (P_iCO_2) should not be high enough to result in hypercapnia.[6]

Currently, the U.S. Navy places the upper limit for CO_2 levels in rebreather UBA scrubber outflow at 0.5% Surface Equivalent Value (SEV).[8] There is some interest within the Navy to increase the P_ICO_2 limit to 1-2% in an attempt to increase canister duration life.[7,9] Allowing 1.0% SEV increase could increase the stated scrubber duration by 15 to 20%; however, it should be noted that the current limit of 0.5% SEV was established to allow a margin of error in an attempt to minimize any possibility that a diver could face a potentially catastrophic level of CO_2 as a result of the variability encountered in diver physiology, diving apparatus, and diving operations.[7] Increases in P_iCO_2, no matter how minimal, can result in some alterations in cardiorespiratory responses, including an increase in respiratory minute ventilation to compensate for the build-up of CO_2 in the lungs,[10] or failure of that response resulting in hypercapnia.

Research has shown that short-term exposure in a dry environment to CO_2 up to 6% CO_2, regardless of whether exercise was being performed, has little effect on neurocognitive and postural stability (i.e., balance). Sheehy, Kamon, and Kiser exposed individuals to up to 5% CO_2 supplied by an open-circuit breathing system for 16 minutes with 10 minutes of the exposure involving submaximal exercise. They reported no significant effects on neurocognitive performance.[11] Henning et al. exposed individuals to 6% CO_2 in 21% O_2 (balance nitrogen) for 5-7 minutes supplied by an open-circuit breathing system followed by 6% CO_2 (balance Oxygen [O_2]) for 10-12 minutes involving no exercise and reported no significant effect on neurocognitive performance, hand steadiness, and postural sway.[12,13] Selkirk et al. exposed submerged Navy Divers 1.5% and 3% CO_2 SEV supplied by an open-circuit system for 30 minutes involving mild- and moderate-intensity exercise and reported depressed neurocognitive performance as measured by tests of long term memory.[14] Vercruyssen et al. reported that breathing up to 4% CO_2 supplied by an open-circuit system in a dry laboratory for 45 minutes with two 15 minute periods of intermittent exercise does not significantly affect neurocognitive and postural sway.[15] Sayers et al exposed individuals to up to 7.5% CO_2 for periods of 20 minutes and 6.5% CO_2 for 80 minutes (prolonged exposure) involving no exercise and reported some significant effects on neurocognitive performance in both short-term exposures > 6.5% and for the prolonged exposures.[16] Finally, Vercruyseen exposed individuals to 4% CO_2 (with 50% O_2) for 60 minutes and reported deficits in neurocognitive functioning as measured by stimulus encoding and response selection.[17]

Taking into account the aforementioned results, it is feasible that prolonged exposures to 1.5% inspired CO_2 even without exercise and/or breathing resistance may result in some reductions in neurocognitive performance as result of impaired vision, diminished motor control, slowed reaction and response time, disorientation, and/or reduced attentional capacities.[15,17] It should be noted that each of the referenced reports focused on open-circuit breathing systems which have varying levels of breathing resistance depending on the design and minute ventilation. Whereas some closed systems, such as rebreather UBAs, may have higher breathing resistances and may result in increased CO_2 retention and higher levels of end tidal CO_2 partial pressure ($P_{ET}CO_2$) especially during periods of exercise.[18-20] Unfortunately, there is a dearth of literature to whether UBAs with high breathing resistance adversely affects neurocognitive and/or motor functioning. It is however, feasible that any UBA with high breathing resistance may result in observable reductions in neurocognitive and motor functioning especially if the diver is breathing high CO_2 levels during periods of exercise.

This report presents some results of NAVSEA task 12-04, "CO_2 and UBA-Like Resistance Underwater: Effects on Exercise Endurance and Cognition."[21] In this study U.S. Navy Divers breathed oxygen underwater, for a mildly hyperoxic oxygen partial pressure of approximately 1.3 atm (130 kPa). P_iCO_2 was increased for some dives, and respiratory resistance that was modeled on the MK 16 UBA. Divers performed in-water endurance exercise at nominally 85% of their peak oxygen uptake rates (85% $\dot{V}O_{2\ peak}$), neurocognitive testing was administered in the water before and after exercise, and balance was assessed after they emerged from the water.

This report concentrates on the data from the neurocognitive and balance testing and their associated measurements. It touches only briefly on the in-water endurance exercise testing to provide a timeline and allow for comparisons to the literature. NEDU TR 14-14[22] presents specifics of the methodology and results of the endurance exercise testing, while NEDU TR 14-02[23] deals with dry testing of resting ventilatory sensitivity to CO_2. It should be noted that the testing performed in the dry laboratory setting was omitted from this TR due to equipment malfunctions.

The hypotheses addressed in this report are that:

1. Neurocognitive performance will be adversely affected by either rebreather-like resistance, inhaled CO_2, or a combination of both;

2. Balance performance will be adversely affected by either rebreather-like resistance, inhaled CO_2, or a combination of both.

METHODS

General

The Institutional Review Board at NEDU approved protocol number 13-11/40052,"CO_2 and UBA-like Resistance Underwater: CO_2 Retention, Cognition, and Exercise Endurance." The participants for this test consisted of sixteen male U.S. Navy divers from NEDU and NEDU Reserve Unit Great Lakes. All participants gave written informed consent before participating in the study. Endurance exercise was imposed five times submerged on the bottom of the NEDU test pool, detailed in Table 1. Specific details pertaining to the exercise testing are given in NEDU TR 14-14[22], but are briefly described here because they were part of the time line of the dives. Neurocognitive testing was performed immediately before and after exercising on the ergometer for each testing session. Balance testing was performed roughly 15 minutes following each post-exercise neurocognitive assessment.

Table 1. Conditions tested

Sessions	Resistance and supplied gas	Assessments
Pre	-	Balance baseline (1), ANAM practice (5)
In-water	R1, 0% SEV CO_2	ANAM, Endurance exercise, ANAM, Balance
	R1, 1% SEV CO_2	ANAM, Endurance exercise, ANAM, Balance
	R1, 2% SEV CO_2	ANAM, Endurance exercise, ANAM, Balance
	R2, 0% SEV CO_2	ANAM, Endurance exercise, ANAM, Balance
	R2, 2% SEV CO_2	ANAM, Endurance exercise, ANAM, Balance

Note. R1: work of breathing per tidal volume (WOB/V_T) =1.0 kPa at a minute ventilation of 62.5 L/min; R2: WOB/V_T = 1.8 kPa at a minute ventilation of 62.5 L/min; SEV = surface equivalent value; CO_2 = carbon dioxide; ANAM = automated neuropsychological assessment metrics.

Neurocognitive Testing

The ANAM was used to assess neurocognitive functioning.[24] The five performance subtests selected from the ANAM battery were: Simple Reaction Time (SRT), Code Substitution (CDS), Code Substitution Delayed (CDD), Switch Tasking (SWT), and Code Substitution Intermediate (CDI). Descriptions of these subtests can be found in Table 2. The ANAM records accuracy, speed, and throughput (TP) performance for each subtest. Throughput is a single outcome measure produced from percent correct (accuracy) divided by mean reaction time (speed). Therefore, TP scores represent the correct number of responses per minute of available response time; thus, higher values indicate better performance. Throughput is considered a measure of effectiveness or cognitive efficiency. TP scores were used in these analyses. Descriptive statistics were computed for the TP of the subtests.

Balance testing

Alterations in postural stability (i.e., balance) were assessed using a Nintendo Wii Balance Board (Nintendo, Kyoto, Japan).[25-29] Custom Matlab (Mathworks, Natick, MA) software was used to conduct the test and collect parameter data sampled at a rate of 30 Hz and filtered with a 9^{th} order Butterworth low-pass filter with a 10 Hz cutoff frequency to isolate the low-frequency postural sway process.

A modified Clinical Test of Sensory Interaction and Balance (CTSIB) was used to assess balance.[30] The assessment included two visual and two task conditions to comprise a total of four conditions. The two visual conditions were eyes open and eyes closed. The two task conditions were a quiet stance and a dual task (standing still plus cognitive task) condition. In the cognitive task, participants verbally counted backwards by 3 from 500 while standing.[31,32] In subsequent trials they continued the subtractions where they previously left off. If five counting errors were made, the task was modified to counting backward by 1 from 500, then counting backward by 3 from 300, then counting backward by 1 from 300, then counting backward by 3 from 100, then counting backward by 1 from 100. Evaluation of cognitive task performance included the number of responses (speed) and the mistakes made by the participant (accuracy) during each trial.

In summary, the conditions were as follows:

1. Eyes open under single task conditions.
2. Eyes closed under dual task conditions.
3. Eyes open under dual task conditions.
4. Eyes closed under single task conditions.

Each assessment consisted of three 70-second trials for each of these four conditions for a total of 12 trials equaling 14 minutes of testing time. Trials were randomly ordered across subjects. Participants sat and rested for 1 minute between trials to reduce fatigue effects. The postural stability parameters (i.e., variables of interest) were amplitude (calculated as standard deviation) and Sample Entropy of the change in center of pressure. Each variable was calculated for the anterior-posterior (y) and medial-lateral (x) time series.

In-water testing

Divers performed endurance exercise testing on five non-consecutive days, 12 feet underwater in a 15 foot deep test pool maintained at 82 ± 5 °F (28 ± 3 °C) with the chlorine levels at 1 to 3 ppm. Participants were asked to avoid caffeine, nicotine, alcohol, decongestants, large meals, and heavy exercise for twelve hours before in-water testing. A different experimental breathing condition was imposed on each of the five days. Breathing gas for all dives was provided by the Test Pool Respiratory Monitoring System (TPRMS), which is detailed in NEDU TR 14-04.[22] The breathing gas provided to the diver was O_2 with one of 0, 1, or 2 kPa CO_2 (0, 1, or 2% SEV CO_2). Dead space in the mask and valves increased the average inhaled PCO_2 by about 0.5 kPa (0.5% SEV).[22] The breathing resistances were imposed such that the total work of breathing per tidal volume (WOB/V_T) was either 1.0 kPa (R1) or 1.8 kPa (R2) at a minute ventilation of 62.5 L/min. Thus, in summary, the five experimental breathing conditions are as follows (breathing resistance, supplied gas):

1. R1, 0% SEV CO_2
2. R1, 1% SEV CO_2
3. R1, 2% SEV CO_2
4. R2, 0% SEV CO_2
5. R2, 2% SEV CO_2

The order of the breathing conditions was a predetermined random order to different divers, and divers were not informed of the order the conditions until after their fifth dives.

During the in-water testing, the diver equilibrated to the test breathing conditions for five minutes at rest on the prone cycle ergometer before completing the neurocognitive testing which lasted an additional 12 to 20 minutes. When the diver finished neurocognitive testing, he was instructed to begin exercise and to maintain a cadence of 60 ± 3 rpm as shown on a display. Exercise began with a three minute warm up with loadless pedaling followed by a pre-determined endurance load (85% $VO_{2\ peak}$) that was based on the results of the dry endurance testing.

Exercise continued for a maximum of 60 minutes (57 minutes at load), or until the diver could not maintain cadence, wished to stop, reported clinical symptomology suggestive of CNS oxygen toxicity or of hypercapnia (i.e., limb convulsions, nausea, dizziness, visual disturbance, facial twitching, confusion, weakness, severe headache, flushed feeling, shortness of breath, or anxiety), or was told to stop by an investigator, research monitor, or dive supervisor. $P_{ET}CO_2$ of 65 Torr (9 kPa) or more for than five consecutive breaths caused a safety abort. Upon stopping the endurance exercise, the duration of exercise was recorded, the ergometer load was removed, and the diver pedaled without added load and with freely chosen cadence for at least 2 and at most 5 minutes (i.e., cooled down). Post exercise neurocognitive testing was completed after the cool down while participants remained on the ergometer and continued to breathe the assigned condition. After neurocognitive testing, participants were instructed to switch to air and surface. After at least ten minutes on the pool deck, participants performed balance testing. Divers who terminated exercise due to severe symptoms or because of a safety abort were immediately switched to auxiliary air and were either instructed to or chose to surface without completing neurocognitive testing.

Data analysis

All participants exercised under all conditions, serving as their own controls for repeated measures or paired statistical tests. In-water breathing conditions were presented in randomized order. The following breathing condition, R1, 0% SEV CO_2, was used as the baseline value for all analyses. If characteristic clinical symptoms associated with CO_2 exposure were present at the time of post-exercise neurocognitive testing was used as a bivariate variable in these analyses.

Neurocognitive testing

Exclusion criteria were applied as follows: invalid pre/post data due to early termination, unpaired pre/post exercise data, missing data from one or more of the subtests, and if TP scores were determined to be extreme outliers as defined as three times the interquartile range above the third quartile and below the first quartile.

After data exclusion, descriptive statistics were computed for TP for each the ANAM performance subtests. The normality of average throughput scores was statistically verified with both Lilliefors and Jarque-Bera tests ($p > 0.05$). Pearson product-moment correlation coefficients were computed between symptoms and throughput scores for each subtest.

A general linear (GLM) model (2x5) multivariate analysis of variance (GLM MANOVA) with repeated measures, with time pre-post exercise (2 levels) and breathing condition (5 levels) as within subjects variables was conducted to evaluate the effect of exercise and breathing conditions on ANAM performance in the water. The main effects of group and time, and the interaction between group and time were tested using the multivariate criterion of Wilk's lambda. Univariate tests were conducted as within-subject planned comparisons. Tests of simple effects were conducted to follow up significant main effects and interactions as needed.

Balance testing

The center of pressure (COP) motion along the anterior-posterior (X) and medio-lateral (Y) axes of motion were obtained from the custom MATLAB software for analysis. COP regularity was quantified using the sample entropy (SampEn) analysis.[33,34] SampEn calculates the probability that a data set of length N that having repeated itself for a window length m within a tolerance of r, will also repeat itself for $m + 1$ data points, without allowing self-matches. SampEn values were calculated for all postural parameters using MATLAB m.files obtained from Physionet (http://www.physionet.org/physiotools/).[35] The parameter values $m = 3$ and $r = 0.3$ were used in the calculations. Center of pressure data exceeding 2.5 standard deviations from the mean of each breathing and postural condition were removed.

Four GLM (4 x 5) MANOVAs with repeated measures, with postural condition (4 levels) and breathing condition (5 levels) as within subjects variables, were conducted to evaluate the effect of breathing conditions on amplitude and SampEn in both the X and Y direction. Univariate tests and simple effects tests were conducted to follow up significant main effects and interactions as needed.

RESULTS

A total of 16 male divers participated in this study. The mean age was 34 years (standard deviation [SD] 7.5). The mean height was 178 cm (SD 3.4). The mean body mass was 86 kg (SD 23.8). Symptoms reported by divers for each condition are listed in Table 2.

Table 2. Reported characteristic clinical symptoms associated with CO_2 exposure (from reference 22)

Condition	Clinical symptoms	N	%
R1, 0% SEV CO_2	Headache	2	13%
	Agitated	1	6%
	Nausea	1	6%
	Dizziness	1	6%
R1, 1% SEV CO_2	Headache	4	25%
	Nausea	2	13%
	Dizziness	1	6%
R1, 2% SEV CO_2	Headache	6	38%
	Nausea	1	6%
	Concentration issues	1	6%
	Sleepy	1	6%
	Dizziness	1	6%
R2, 0% SEV CO_2	Nausea	1	6%
	Tunnel vision	1	6%
	Anxious	1	6%
	Concentration issues	1	6%
	Hot	1	6%
	Light-headed	1	6%
R2, 2% SEV CO_2	Headache	7	44%
	Flushed	1	6%
	Feeling ‘loopy’	1	6%
	Agitated	1	6%
	Nausea	2	13%
	Anxious	3	19%
	Confused	1	6%
	Hot	1	6%
	Light-headed	1	6%
	Dizziness	1	6%

Note. R1: work of breathing per tidal volume (WOB/V_T) =1.0 kPa at a minute ventilation of 62.5 L/min; R2: WOB/V_T = 1.8 kPa at a minute ventilation of 62.5 L/min; SEV = surface equivalent volume; CO_2 = carbon dioxide; N = number of divers; % = percentage of divers.

Neurocognitive testing

Descriptive statistics of throughput scores for each ANAM subtest can be found in Tables 3. The number of participants who completed each ANAM subtest for all breathing conditions is also tabulated. Please note that, as reflected by N<16, not all participants were able to complete ANAM testing for all breathing conditions due to either early termination of dives (usually because of severe symptoms or abort-level end-tidal CO_2) or ANAM equipment malfunction.

Table 3. Descriptive statistics for the ANAM subtests

Subtest	Breathing Condition	N	Pre-		Post-	
			Mean	Stdev	Mean	Stdev
CDS	R1, 0% SEV CO_2	12	43.18	14.00	44.48	15.23
	R1, 1% SEV CO_2	15	46.41	12.83	42.30	12.58
	R1, 2% SEV CO_2	12	45.50	9.45	45.62	8.35
	R2, 0% SEV CO_2	15	50.26	9.62	47.03	15.16
	R2, 2% SEV CO_2	9	43.60	10.27	42.95	11.28
CDD	R1, 0% SEV CO_2	12	53.37	20.30	47.92	17.73
	R1, 1% SEV CO_2	15	50.82	11.64	43.95	12.97
	R1, 2% SEV CO_2	12	53.70	12.42	45.62	11.02
	R2, 0% SEV CO_2	15	55.53	13.67	52.30	14.80
	R2, 2% SEV CO_2	9	49.09	15.52	47.86	12.72
CDI	R1, 0% SEV CO_2	12	53.22	16.06	47.40	15.23
	R1, 1% SEV CO_2	15	49.21	12.02	43.85	11.52
	R1, 2% SEV CO_2	12	50.86	12.09	45.62	11.55
	R2, 0% SEV CO_2	15	55.11	14.63	50.50	15.43
	R2, 2% SEV CO_2	9	45.23	14.15	50.24	15.61
SWT	R1, 0% SEV CO_2	12	23.28	7.09	25.42	8.80
	R1, 1% SEV CO_2	15	27.43	6.81	27.45	7.27
	R1, 2% SEV CO_2	12	23.99	7.50	45.62	6.71
	R2, 0% SEV CO_2	15	26.18	5.81	26.58	5.72
	R2, 2% SEV CO_2	9	23.04	8.07	26.04	7.30
SRT	R1, 0% SEV CO_2	12	190.55	19.32	203.18	27.87
	R1, 1% SEV CO_2	15	175.86	37.92	186.80	30.39
	R1, 2% SEV CO_2	12	192.64	32.23	186.90	37.75
	R2, 0% SEV CO_2	15	191.04	30.42	181.04	25.31
	R2, 2% SEV CO_2	9	184.81	25.07	180.27	21.18
SRT2	R1, 0% SEV CO_2	12	183.46	20.65	193.54	24.33
	R1, 1% SEV CO_2	15	178.55	38.97	180.20	37.04
	R1, 2% SEV CO_2	12	178.24	23.61	186.90	33.17
	R2, 0% SEV CO_2	15	189.63	35.91	185.57	26.55
	R2, 2% SEV CO_2	9	179.31	23.65	189.71	17.23

Note. N = sample size; CDS = code substation; CDD = code substitution delayed; CDI = code substitution intermediate; SWT = switching task; SRT = simple reaction time; SRT2 = 2^{nd} simple reaction time test; R1: work of breathing per tidal volume (WOB/V_T) =1.0 kPa at a minute ventilation of 62.5 L/min; R2: WOB/V_T = 1.8 kPa at a minute ventilation of 62.5 L/min; SEV = surface equivalent volume; CO_2 = carbon dioxide; stdev = standard deviation. All values in are throughput (correct responses/minute).

There was no correlation between the symptomology and CDS (r = -.061, n = 63, p = .637), CDD (r = -.113, n = 63, p = .377), CDI (r = -.023, n = 63, p = .860), SWT (r = .136, n = 63, p = .287), SRT (r = -.208, n = 63, p = .101), and SRT2 (r = -.100, n = 63, p = .436).

The results of the GLM MANOVA with repeated measures revealed that there was no significant interaction between time pre- or post-exercise and breathing condition (λ = .378, $F_{(24,67.493)}$ = .904, p = .596). There was also no main effect for time (λ = .507, $F_{(6,1)}$ = 1.62, p = .596) or breathing condition (λ = .360, $F_{(24,67.493)}$ = .956, p = .531).

The results of the planned univariate effects tests for time revealed that there was no main effect for CDS ($F_{(1,6)}$ = .621, p = .461), CDD ($F_{(1,6)}$ = .305, p = .601), CDI ($F_{(1,6)}$ = .472, p = .518), SWT ($F_{(1,6)}$ = .133, p = .728), SRT ($F_{(1,6)}$ = 1.278, p = .301), and SRT2 ($F_{(1,6)}$ = .140, p = .721).

The results of the planned univariate tests for breathing condition revealed that there was a significant main effect for SRT ($F_{(4,24)}$ = 3.375, p = .025); however, post-hoc pairwise comparisons revealed no further significance. There were no main effects for CDS ($F_{(4,24)}$ = .918, p = .470), CDD ($F_{(4,24)}$ = .367, p = .829), CDI ($F_{(4,24)}$ = 1.502, p = .233), SWT ($F_{(4,24)}$ = .401, p = .806), and SRT2 ($F_{(4,24)}$ = .442, p = .77).

The results of the planned contrast for the interaction of time and breathing condition revealed that there was a significant interaction for SWT ($F_{(4,24)}$ = 2.992, p = .039); however, tests of simple effects revealed no further significance.
There were no significant interactions for SRT ($F_{(4,24)}$ = .027, p = .998), and no main effect for CDS ($F_{(4,24)}$ = .853, p = .506), CDD ($F_{(4,24)}$ = 1.683, p = .187), CDI ($F_{(4,24)}$ = .295, p = .878), and SRT2 ($F_{(4,24)}$ = .782, p = .548).

Nevertheless, there was however a noticeable trend in the data as there was a mean non-significant decrease in neurocognitive performance for all subtests following the endurance exercise testing regardless of the breathing condition.

Balance testing

Descriptive statistics for COP amplitude in the X and Y-direction and COP SampEn in the X and Y-direction can be found in Tables 4 and 5, respectively.

The results of the ANOVA for COP amplitude in the X-direction revealed that there was no significant interaction between breathing condition and postural assessment condition ($F_{(15,225)}$ = .885, p = .582, partial η^2= .056). The main effects for breathing condition ($F_{(5,75)}$ = .794, p = .558, partial η^2= .050) and postural assessment condition ($F_{(3,45)}$ = .694, p = .561, partial η^2= .044).

The results of the ANOVA for COP displacement amplitude in the Y-direction revealed that there was no significant interaction between breathing condition and postural assessment condition ($F_{(15,225)}$ = .941, p = .520, partial η^2= .059). The main effects for breathing condition ($F_{(5,75)}$ = .064, p = .997, partial η^2= .004) and postural assessment condition ($F_{(3,45)}$ = .230, p = .875, partial η^2= .015).

Table 4. Average standard deviation of center of pressure position

Direction	Breathing Condition	Amplitude (mm)							
		EOST		ECDT		EODT		ECST	
		M	SD	M	SD	M	SD	M	SD
X	BSL	0.27	0.11	0.3	0.16	0.34	0.29	0.32	0.11
	*R*1, 0% SEV CO_2	0.26	0.09	0.25	0.1	0.28	0.08	0.26	0.11
	*R*1, 1% SEV CO_2	0.32	0.19	0.27	0.07	0.49	0.95	0.27	0.07
	*R*1, 2% SEV CO_2	0.28	0.08	0.28	0.11	0.32	0.2	0.29	0.12
	*R*2, 0% SEV CO_2	0.27	0.10	0.37	0.24	0.25	0.08	0.26	0.1
	*R*2, 2% SEV CO_2	0.34	0.22	0.31	0.19	0.26	0.08	0.3	0.12
Y	BSL	0.55	0.17	0.65	0.32	0.65	0.35	0.62	0.19
	*R*1, 0% SEV CO_2	0.58	0.26	0.68	0.28	0.6	0.27	0.6	0.16
	*R*1, 1% SEV CO_2	0.69	0.43	0.57	0.17	0.62	0.43	0.59	0.16
	*R*1, 2% SEV CO_2	0.6	0.17	0.6	0.22	0.61	0.23	0.61	0.18
	*R*2, 0% SEV CO_2	0.6	0.22	0.63	0.17	0.6	0.22	0.59	0.17
	*R*2, 2% SEV CO_2	0.64	0.18	0.63	0.33	0.58	0.24	0.6	0.18

Note. X = medial-lateral; Y= anterior-posterior R1: work of breathing per tidal volume (WOB/V_T) =1.0 kPa at a minute ventilation of 62.5 L/min; R2: WOB/V_T = 1.8 kPa at a minute ventilation of 62.5 L/min; SEV = surface equivalent volume; CO_2 = carbon dioxide; EOST = eyes open single task; EODT = eyes open dual task; ECST = eyes open single task; ECST = eyes closed dual task; M = mean; SD = standard deviation.

Table 5. Average sample entropy or center of pressure regularity.

Direction	Breathing Condition	Sample Entropy							
		EOST		ECDT		EODT		ECST	
		M	SD	M	SD	M	SD	M	SD
X	BSL	.32	.15	.34	.10	.32	.10	.28	.07
	*R*1, 0% SEV CO_2	.32	.10	.38	.10	.31	.08	.34	.10
	*R*1, 1% SEV CO_2	.32	.07	.35	.08	.36	.15	.32	.10
	*R*1, 2% SEV CO_2	.29	.08	.37	.14	.32	.14	.33	.12
	*R*2, 0% SEV CO_2	.30	.10	.32	.11	.35	.13	.37	.10
	*R*2, 2% SEV CO_2	.29	.09	.31	.09	.30	.08	.29	.08
Y	BSL	.45	.17	.52	.15	.44	.12	.49	.11
	*R*1, 0% SEV CO_2	.46	.09	.50	.16	.45	.10	.48	.15
	*R*1, 1% SEV CO_2	.40	.11	.55	.12	.50	.11	.49	.13
	*R*1, 2% SEV CO_2	.42	.08	.53	.11	.46	.09	..49	.10
	*R*2, 0% SEV CO_2	.40	.12	.50	.13	.43	.11	.49	.13
	*R*2, 2% SEV CO_2	.39	.14	.52	.12	.44	.11	.48	.14

Note. X = medial-lateral; Y= anterior-posterior; R1: work of breathing per tidal volume (WOB/V_T) =1.0 kPa at a minute ventilation of 62.5 L/min; R2: WOB/V_T = 1.8 kPa at a minute ventilation of 62.5 L/min CO_2 = carbon dioxide; EOST = eyes open single task; EODT = eyes open dual task; ECST = eyes open single task; ECST = eyes closed dual task; M = mean; SD = standard deviation.

The results of the ANOVA for COP SampEn in the X-direction revealed that there was no significant interaction between breathing condition and postural assessment condition ($F_{(15,225)}$ = 1.294, p = .2.07, partial η^2= .079). The main effects for breathing condition was also revealed to be not significant ($F_{(5,75)}$ = 1.563, p = .181, partial η^2= .094).

The results of the ANOVA for COP SampEn in the Y-direction revealed that there was no significant interaction between breathing condition and the postural assessment condition ($F_{(15,225)}$ = 1.01, p = .448, partial η^2= .063). The main effects for breathing condition was also revealed to be not significant ($F_{(5,75)}$ = .640, p < .001, partial η^2= .386).

DISCUSSION

This effort examined the effect of varying levels of CO_2 exposures and breathing resistance underwater on neurocognitive performance and postural stability (i.e., balance). The primary findings from this study were that there were no statistically significant differences between baseline neurocognitive and balance performance and post-exposure performance for any of the breathing conditions. In the subjects tested, no adverse effects of mean neurocognitive performance or postural stability were detected after exposures to up to 2%SEV CO_2 with resistance to impose WOB/VT as high as 1.8 kPa at 62.5 L/min. Unfortunately, subjects with severe symptoms in the water and those who reached abort-level end tidal CO_2 were not tested. Further, it should be noted that at least three different (Table 2) clinical symptoms associated with CO_2 exposures were reported for each condition. A complete description of the symptomology can be found in NEDU TR 14-14.

These results agree with the existing literature that has reported that acute exposures (i.e., ~ 60 minutes) to CO_2 has little effect on neurocognitive and postural stability (i.e., balance). The data were examined individually to determine if any of the divers deviated from the post-dive group means and there were no outliers. One can assume that the divers who had an abort during the high CO_2 conditions (due to clinical symptomology or high $P_{ET}CO_2$) would have had large decreases in post-exercise performance compared to the group mean. It is feasible that these hypothetical decreases would be a result of fatigue and mild dehydration following the immersion and exercise.[36-39]

There were limitations in this study that may have affected the results. They are listed here for easy reference.

1. The individuals who were most likely to have shown neurocognitive effects from the CO_2 exposures did not complete post-exercise neurocognitive assessments.

2. A safety period of ten minutes (i.e., clean time) was used to determine if the divers were neurologically sound prior to the balance testing. This clean time may have allowed ample time for participants to recover from the effects of CO_2. It is possible that if the balance testing is performed during clean time the assessment may be able to detect subtle differences in postural stability prior to recovery.

3. The selected ANAM subtests and postural assessment may not have been sensitive enough to elicit subtle decrements in neurocognitive performance and postural stability, respectively.

Perhaps due to the lack of significant differences in cognitive performance across breathing conditions, a more comprehensive neuropsychological assessment performed by a licensed neuropsychologist could be explored for future studies. Additionally, a more challenging assessment of neuromotor functioning, such as an assessment of gait over uneven terrain, should be explored as well.

CONCLUSIONS

The results of this study can be interpreted as indicating that the various combinations of high CO_2 levels and high breathing resistance did not affect average neurocognitive performance and postural stability. Affirming the null hypothesis and suggesting that there was not an effect due to the breathing conditions is still not grounds for raising the upper limit for P_ICO_2 levels especially in the presence of clinical symptoms. It simply means that under the breathing conditions specified in this report, the testing measures chosen failed to show sensitivity to the combination of breathing resistance and CO_2. There is still a possibility that different dependent variables, more sensitive testing measures, longer exposures, higher resistance levels, or combinations of each might result in a significant effect at 2% SEV or lower CO_2.

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